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RELATION OF TEMPERATURE TO INFECTION OF BEAN AND COWPEA SEEDLINGS BY RHIZOCTONIA BATATICOLA[1]

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KENDRICK(6) HAS DESCRIBED a serious seedling blight of beans (*Phaseolus vulgaris* L.) occurring in the Sacramento Valley and delta region, caused by the fungus, *Rhizoctonia bataticola* (Taub.) Butler. He showed that the disease was favored by high temperatures during the period of seedling emergence and observed that cowpeas (*Vigna sinensis* L.) under similar conditions appeared to escape infection. In order to learn more about the temperature relations, the mode of infection, and the apparent resistance of cowpeas, cultures of *Rhizoctonia bataticola* (Taub.) Butler isolated from various hosts have been compared as to cultural characters and effect of temperature on growth and pathogenicity to bean and cowpea seedlings.

SOURCES OF CULTURES

The cultures of *Rhizoctonia bataticola* from beet were isolated from a root-rot of sugar beet—A, B, and C, from beets collected near Walnut Grove, California; D and E near Marysville; and F near Stockton. The cultures from bean (A, B, C) were isolated by J. B. Kendrick from bean seedlings—A and B at Davis, C near Lodi. The culture from cowpea was isolated from herbarium specimens of older diseased Blackeye cowpea plants collected at Modesto, California, by W. W. Mackie. The two cultures from sweet potato were isolated from a rot of sweet potatoes near Atwater, California. The culture from begonia was supplied by M. R. Harris of the California State Department of Agriculture. The culture from citrus was supplied by H. S. Fawcett, Riverside. The

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culture from strawberry was isolated by W. C. Snyder from strawberry roots from Santa Cruz, and the culture from cotton was by Snyder from specimens sent from Texas by J. J. Taubenhaus. Haigh's A, B, and C cultures were kindly sent to Kendrick by J. C. Haigh. Culture A was from *Acacia* in Kenya, B from hibiscus in Ceylon, and C from *Juniperus* in Ceylon. A culture of *Rhizoctonia solani* isolated by L. D. Leach from dry rot of sugar beet at Davis, was also used.

MORPHOLOGICAL AND CULTURAL CHARACTERS OF THE CULTURES USED

In an attempt to bring about the production of pycnidia, all except the culture from cowpea were grown on sterilized beet plugs, potato plugs, and bean pods at 28°, 31°, and 34°C for a month and then at room temperatures until dried out, but no pycnidia were produced; nor were they produced in cultures on Leonian's agar,[7] Coon's agar,[3] Brown's asparagin agar,[2] Dox's agar as used by Haigh,[4] cornmeal agar, or prune agar.

No two of the cultures were alike in cultural characters even though six were from sugar-beet roots, three from bean seedlings, and two from sweet potatoes. The cultures were grown on cornmeal agar, potato dextrose agar, prune agar, and Dox's agar in petri plates at 28°C and compared at the end of four days as to color and type of mycelium and abundance of sclerotia, but no grouping was possible. The mycelial differences in seven of the cultures grown on prune agar for two days at 28°C are shown in figure 1. With all of the cultures, potato dextrose agar was most favorable and Dox's agar was least favorable for the production of sclerotia.

The cultures were compared as to chromogenesis in tube cultures on Brown's asparagin agar, Coon's agar, Leonian's agar, and potato dextrose agar at the end of two months. In general, there was no discoloration of the agar. However, in Brown's asparagin agar, Haigh's A strain produced a light brown color; and a pinkish color was produced by the B culture from bean and the cultures from strawberry in all of the media, by the B culture from beet in all except Leonian's agar, by the D culture from bean in Leonian's agar and by the culture from cotton in Leonian's and potato dextrose agar.

All of the cultures with the exception of Haigh's A and B strains, which have large sclerotia, were compared as to size of sclerotia in cultures grown 14 days at 28°C on potato dextrose agar of pH 5.6. One hundred sclerotia of each culture were measured and the mean diameters in microns were as follows: Beet A, 81.9; beet B, 83.8; beet C, 73.8; beet D, 86.8; beet E, 86.8; beet F, 87.2; bean A, 81.9; bean B, 75.4; bean C,

109.7; cowpea, 121.6; sweet potato A, 129.8; sweet potato B, 125.1; begonia, 108.9; citrus 66.2; strawberry, 110.1; cotton, 71.9; Haigh C, 68.7.

All except the cultures from sweet potato and cowpea fall within the limits of Haigh's C group, in which the diameter of the sclerotia is 120μ or less, and, according to the work of Ashby[1] and Haigh,[4] should be designated as *Macrophomina phaseoli* (Maubl.) Ashby. The sclerotia of

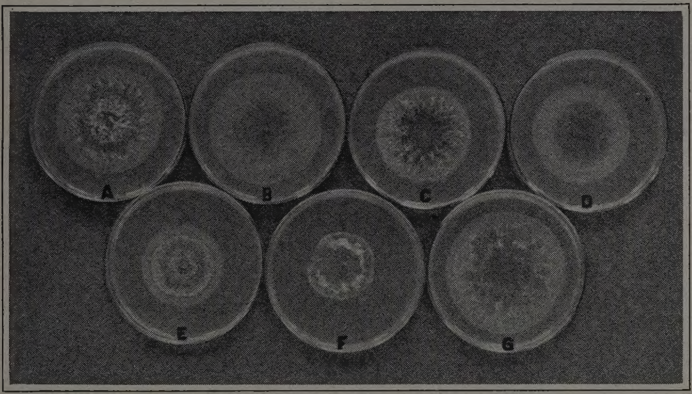

Fig. 1.—Differences in the mycelium of cultures of *Rhizoctonia bataticola;* 2-day old colonies on prune agar, pH 5.6, at 28° C: *A, B, C,* cultures A, B, and D from sugar beets; *D,* culture A from sweet potato; *E,* culture C from Mexican Red bean; *F,* culture from citrus; *G,* culture from strawberry. All except the culture from citrus (*F*) were pathogenic to bean and cowpea seedlings.

the cultures from sweet potato and cowpea were only slightly larger than the limit established for Haigh's C group and are scarcely large enough to warrant classification in the B group in which Haigh gives the diameter of the sclerotia as about 200μ. The culture of Haigh's A strain, with large sclerotia, according to the work of Hopkins,[5] should be designated as *Rhizoctonia lamellifera* Small.

EFFECT OF TEMPERATURE ON GROWTH OF MYCELIUM IN CULTURE

Eight cultures of the fungus were grown at controlled temperatures ranging from 3° to 40°C. Large test tubes provided with a dam at the open end, made by heating the glass and indenting one side, were used in determining the rate of mycelial growth. Prune agar (15 cc) was placed in each tube and allowed to cool with the tube in a horizontal position, the dam preventing the escape of the melted agar. When solidified the agar extended the entire length of the tube (20 cm) and was

uniform in depth. Each tube was inoculated near the dam at the open end with aerial mycelium from plate cultures, incubated 24 hours at room temperature, and then marked with a wax pencil to indicate the edge of the colony.

Three tubes of each culture were then placed in each incubator in a horizontal position and the amount of mycelial growth beyond the first

TABLE 1

RELATION OF TEMPERATURE TO RATE OF MYCELIAL GROWTH OF CULTURES OF RHIZOCTONIA BATATICOLA FROM DIFFERENT HOSTS

Temperature, °C	Average daily growth of mycelium, in mm							
	Beet A	Bean C	Cowpea	Sweet potato B	Begonia	Citrus	Straw-berry	Cotton
3	0.0	0.0	....	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0
12	3.6	3.4	5.3	3.3	3.4	0.7	4.8	3.7
16	11.0	10.6	13.3	11.9	13.0	4.4	12.5	13.2
19	12.6	11.3	11.5	13.2	14.4	4.6	13.7	13.5
22	15.6	11.2	16.5	14.9	16.3	7.5	17.3	17.3
25	25.3	20.0	28.7	23.7	29.8	13.1	27.4	30.8
28	27.4	21.0	29.1	25.8	29.0	_14.8_*	29.3	31.3
31	_31.0_	22.3	32.2	_26.4_	_32.0_	14.4	_32.0_	_33.3_
34	28.8	_26.4_	_37.3_	26.0	30.0	6.5	30.3	32.5
37	17.5	18.3	29.0	4.8	2.7	1.6	23.0	15.4
40	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.3

* Figures in italics indicate the growth at the optimum temperature in culture.

wax pencil mark was measured daily until the mycelium had grown about 12 to 14 cm. The daily rates of growth at each temperature were fairly uniform, but in most instances the maximum occurred on the first or second day. The average daily growth of each culture is recorded in table 1.

The results, summarized in table 1, show that practically no growth occurred at 3° or 8°C and very little at 12°, and that the optimum temperature was about 31° for all except the cultures from bean and cowpea, which grew most rapidly at 34°, and the culture from citrus, which grew only about half as fast as the others and proved to be practically nonpathogenic. The culture from cotton was the only one to grow at 40°, and was the only one of these eight cultures found to cause infection at this temperature.

METHOD OF TESTING PATHOGENICITY OF FUNGUS ON SEEDLINGS

In testing the pathogenicity of the cultures, seeds of Mexican Red bean (*Phaseolus vulgaris* L.) and California Blackeye cowpea (*Vigna sinensis* L.), which had been surface-sterilized in mercuric chloride 1:1000,

TABLE 2

INFECTION OF MEXICAN RED BEAN SEEDLINGS BY CULTURES OF RHIZOCTONIA BATATICOLA AT DIFFERENT TEMPERATURES

Culture	20-23°		25°		28°		31°		34°		37°		40°	
	Total seedlings	Per cent infected	Total seedlings	Per cent infected	Total seedlings	Per cent infected	Total seedlings	Per cent infected	Total seedlings	Per cent infected	Total seedlings	Per cent infected	Total seedlings	Per cent infected
Beet A	40	2	18	33	19	32	37*	51	25	92	27	89	0	....
Beet B	36	11	16	25	18	5	35	34	33	73	26	50	5	20
Beet C	29	10	19	21	15	13	29	55	31	71	17	88	0	....
Beet D	40	25	14	28	7	57	27	52	20	95	26	92	9	66
Beet E	33	15	20	10	14	14	23	69	31	52	34	50	2	50
Beet F	40	7	11	27	17	0	39	33	33	88	35	91	8	25
Bean A	36	17	18	28	15	27	36	42	29	62	18	83	10	50
Bean B	33	12	16	6	16	12	32	22	31	42	39	36	9	11
Bean C	38	13	19	5	17	18	38	18	33*	48	24	62	0	....
Cowpea†	0	....	40	5	44	2	47	10	48*	62	46	61	0	....
Sweet potato A	36	8	17	6	19	31	32	25	33	51	24	33	5	0
Sweet potato B	31	16	16	12	17	35	35*	60	27	85	26	4	0	....
Begonia	33	3	12	8	14	15	26*	69	30	46	21	5	0	....
Citrus	40	0	19	0	19*	0	38	5	36	8	18	11	2	0
Strawberry	34	6	20	5	17	18	28*	18	33	91	21	86	0	....
Cotton	36	0	19	0	20	0	38*	5	32	69	20	55	10	90
Haigh A	29	0	13	0	19	0	38	5	34	0	27	4	15	0
Haigh B	38	0	18	0	19	0	36	3	38	3	39	10	14	0
Haigh C	38	10	18	17	16	0	36	61	27	81	27	67	6	17
Control	107	0	57	0	52	0	102	0	110	0	77	0	12	0
Rhizoctonia solani	16	75	30	33	33	33	25	32	33	15	26	0	1	0

* Optimum temperature for growth of mycelium in culture.

† The tests with the cowpea culture were made in April, 1934.

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rinsed, and dried, were planted in moist sterilized sand with which shredded prune agar plate cultures of the fungus had been mixed. Paraffined cardboard drinking cups, 7 cm deep and 7 cm in diameter, were used as containers. Inoculum was used at the rate of one petri plate culture for two cups, and the moist sand with which the inoculum had been mixed was incubated 4 to 6 days at room temperature before it was placed in the cups. Ten seeds were planted in each cup. Two cups were planted with beans and two with cowpeas for each culture at each temperature. In addition, six cups with sterile prune agar as inoculum were used as controls at each temperature, all of which remained free from infection, and two with a culture of *Rhizoctonia solani*.

Preliminary tests showed that infection occurred most commonly on the cotyledons only, so that it was necessary, in examining the seedlings, to remove the seed coats in order to detect the lesions on the cotyledons. These lesions were at first circular dark-brown or black spots of various sizes (fig. 2 *A, C, D*). Usually these lesions coalesced more or less (fig. 2 *B*) and frequently the entire cotyledon was invaded and blackened (fig. 2 *E, F*) and contained numerous sclerotia. The hypocotyl lesions were linear and blackened (fig. 2 *A, C, F*). The identity of the fungus was proved by numerous reisolations from the cotyledon and hypocotyl lesions.

The composite results of two series of tests with beans are shown in table 2, and two series of tests with cowpeas in table 3. In the first series, the cups were planted and placed in the incubators on February 17, 1933, and removed after 4 days. The temperatures used were 31°, 34°, 37°, 40°, and room temperature (20–23°C). In the other series the cups were planted and placed in the incubators on April 11, 1933. The cowpeas, because of their rapid growth, were removed after 3 days and the beans after 6 days. The temperatures used were room temperature (20°–23°), 25°, 28°, 31°, 34°, 37°, and 40°C. The series at room temperatures were exposed to light during the day. The other temperatures were provided by incubators which were not lighted.

INFECTION OF BEAN SEEDLINGS AT DIFFERENT TEMPERATURES

The results summarized in table 2 show that high percentages of the bean seedlings were infected at 31°, 34°, and 37°C, and that considerable infection occurred at the other temperatures also. All of the cultures were distinctly pathogenic except the citrus and Haigh's A and B, and these cultures differed markedly in other respects as well.

For the eight cultures previously tested for rate of growth at different temperatures, the highest percentage of infection occurred at or above

TABLE 3

INFECTION OF CALIFORNIA BLACKEYE COWPEA SEEDLINGS BY CULTURES OF RHIZOCTONIA BATATICOLA AT DIFFERENT TEMPERATURES

Culture	20-23°		25°		28°		31°		34°		37°		40°	
	Total seedlings	Per cent infected	Total seedlings	Per cent infected	Total seedlings	Per cent infected	Total seedlings	Per cent infected	Total seedlings	Per cent infected	Total seedlings	Per cent infected	Total seedlings	Per cent infected
Beet A	39	0	19	0	18	11	20*	0	34	0	38	0	20	0
Beet B	38	0	19	10	20	5	37	3	38	5	38	0	29	3
Beet C	37	0	20	25	20	15	37	11	35	3	38	0	28	0
Beet D	40	10	20	60	18	61	36	14	25	16	38	3	27	0
Beet E	40	15	19	26	20	10	36	8	37	0	33	0	34	3
Beet F	38	0	19	0	17	6	38	3	38	5	39	3	38	8
Bean A	39	0	19	0	19	5	40	2	39	0	35	0	32	0
Bean B	39	10	19	26	20	35	37	16	37	3	35	6	37	11
Bean C	40	5	19	5	18	11	40	5	38*	0	36	0	18	0
Cowpea†	0	....	44	48	0	....	47	57	46*	72	48	27	0	....
Sweet potato A	40	5	20	0	20	15	39	0	37	0	37	0	19	0
Sweet potato B	40	2	20	20	20	0	39*	0	38	0	33	0	27	0
Begonia	40	0	20	0	20	0	39*	3	40	0	33	0	20	0
Citrus	40	0	18	0	17*	0	40	0	38	0	35	0	36	0
Strawberry	39	2	20	35	17	41	39*	23	38	8	37	3	20	0
Cotton	40	5	20	10	18	17	38*	13	40	0	34	0	27	7
Haigh A	40	0	17	0	19	0	39	0	37	0	36	0	36	0
Haigh B	40	0	20	0	20	5	38	0	37	0	37	0	38	0
Haigh C	40	0	20	10	19	16	39	0	39	0	37	0	20	0
Control	114	0	60	0	49	0	115	0	111	0	111	0	73	0
Rhizoctonia solani	34	35	20	65	17	117	37	8	36	0	35	0	37	3

* Optimum temperature for growth of mycelium in culture.

† The tests with the cowpea culture were made in April, 1934.

370
−117
253

166
158

273

the optimum temperature for mycelial growth, but the culture from cotton, the only one to grow at 40°, was the only one of the eight to infect at this temperature. However, seven cultures not included in the rate-of-growth tests were pathogenic at 40°. The fungus was reisolated from 424 of the bean seedlings. As was to be expected, *Rhizoctonia solani* was more pathogenic at the lower temperatures.

INFECTION OF COWPEA SEEDLINGS AT DIFFERENT TEMPERATURES

The cowpea seedlings, as shown in table 3, were infected much less abundantly than the beans at the higher temperatures where the beans had proved most susceptible. In fact, there was little or no infection of the cowpeas at 34°, 37°, and 40°C, except with the culture from cowpea, while at 25° and 28° the cowpeas were only slightly less susceptible than the beans. With cowpeas, therefore, the maximum infection with all except the culture from cowpea occurred at temperatures (25° and 28°) somewhat below the optimum for growth of the fungus in culture (31° and 34°) but nevertheless within the range of temperatures favoring very rapid mycelial growth. The fungus was reisolated from 110 of the cowpea seedlings. *Rhizoctonia solani* was pathogenic to cowpea seedlings at the lower temperatures.

With the exception of the culture from begonia, the cultures which were pathogenic to the beans were also pathogenic to the cowpeas, although the A and F cultures from beet and the A culture from bean were less active. The culture from cowpea, which presumably is the same fungus which Mackie[8] has found very destructive in the Blackeye variety in the San Joaquin Valley when the plants are older, differed from all of the other cultures in being distinctly pathogenic at all temperatures tested (25°, 31°, 34°, 37°C).

SEEDLING INFECTION COURTS

Of a total of 932 infected bean seedlings, exclusive of those infected by the cowpea culture, 84 per cent were infected only in the cotyledons (fig. 2*B*) and but 9 per cent in the hypocotyl alone. Of the 182 infected cowpea seedlings, exclusive of those infected by the culture from cowpea, 82 per cent were infected only in the cotyledons and 13 per cent only in the hypocotyl. With the culture from cowpea there was much more hypocotyl and epicotyl infection in cowpea seedlings at the higher temperatures. With *Rhizoctonia solani*, 21 per cent of the infected beans and 50 per cent of the infected cowpeas were infected in the cotyledons.

The preponderance of cotyledon infection may explain why so much of the field infection observed by Kendrick[6] was in the form of lesions

at the cotyledonary node. In fact, in many of the bean seedlings the infection had already progressed apparently from the cotyledons into the upper part of the hypocotyl (fig. 2 *E*).

In accord with Kendrick's field observations, no direct root infection was found. Consequently it would seem that seedling infection in the field would be most favored by the occurrence of high soil temperatures prior to the emergence of the cotyledons from the soil.

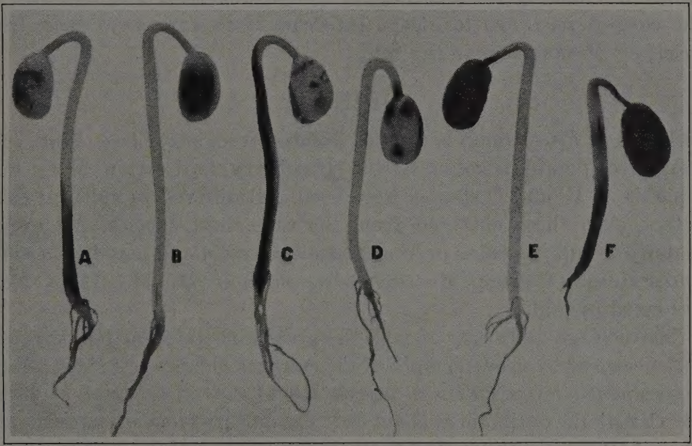

Fig. 2.—Cotyledon and hypocotyl infection of Mexican Red bean seedlings grown in sand inoculated with *Rhizoctonia bataticola: A*, small lesions on cotyledon and large lesion at base of hypocotyl; *B*, coalescence of cotyledon lesions; *C*, small lesions on cotyledon and lesion extending nearly the whole length of the hypocotyl; *D*, larger, circular lesions on cotyledon; *E*, cotyledons completely blackened and invasion extending into upper part of hypocotyl; *F*, cotyledons and lower and upper parts of hypocotyl infected.

RATE OF GROWTH OF SEEDLINGS IN RELATION TO INFECTION

It seems possible that the effect of different temperatures upon the rate of growth of the seedlings may explain why the cowpeas tended to escape infection at the higher temperatures. It was noted that at the higher temperatures some of the infected beans germinated but failed to emerge from the sand and at 40°C many failed to germinate, while the cowpeas germinated vigorously at all of the temperatures.

In a preliminary test of the effect of temperature on rapidity of germination and seedling growth it was found that the cowpea seedlings emerged more promptly than the beans at 28°, 31°, 34°, and 37°C, and in average length of hypocotyl at 3 days exceeded the beans by 0.8 cm

at 28°, 1.4 cm at 31°, 3.4 cm at 34°, and 4 cm at 37°. The cowpea hypocotyls averaged 3.6 cm at 28°, 5.4 cm at 31°, 6.7 cm at 34°, and 5.6 cm at 37°. Elongation of the bean hypocotyls was greatly retarded at 37°. Since most of the infection by all except the culture from cowpea took place in the cotyledons, it seems possible that the more rapid emergence and growth of the cowpea seedlings, by promptly removing the cotyledons from contact with the inoculum in the soil, may be a factor in enabling the seedlings to escape infection. The tendency of the cowpea seedlings to escape infection is in agreement with Kendrick's[6] observations in the field.

SUMMARY

Cultures of *Rhizoctonia bataticola* isolated from sugar beet, bean, cowpea, sweet potato, begonia, citrus, strawberry, and cotton, along with Haigh's A, B, and C strains were used. All differed in cultural characters, even those obtained from the same host species. All except Haigh's A and B strains produced small sclerotia and may be classified under Haigh's C group, *Macrophomina phaseoli* (Maubl.) Ashby. None produced pycnidia.

The average daily rate of mycelial growth at different temperatures was measured by growing eight of the cultures in large test tubes affording an agar surface 20 cm in length. Rapid growth occurred at 25° to 34° C, with the optimum at about 31°. The culture from citrus grew only about half as rapidly as the others, had a lower optimum temperature, and proved to be nonpathogenic.

In testing the pathogenicity of the cultures at different temperatures, Mexican Red bean and California Blackeye cowpea seeds were planted in moist inoculated sand in paraffined cardboard cups and germinated at different temperatures (20–23°, 25°, 28°, 31°, 34°, 37°, and 40°C).

High percentages of the bean seedlings were infected at 31°, 34°, and 37°, and considerable infection occurred at all temperatures. All the cultures were pathogenic except the citrus and Haigh's A and B.

With the exception of the begonia culture, the cultures which were pathogenic to beans also were pathogenic to cowpeas, although some differences in degree were noted.

The culture from cowpea was distinctly the most pathogenic to cowpea seedlings, particularly at the higher temperatures.

The cowpea seedlings tended to escape infection with all except the culture from cowpea at 34°, 37°, and 40°C, but at 25° and 28° were nearly as susceptible as the beans.

Most of the infection of both beans and cowpeas occurred in the cotyledons.

The relative freedom from infection of the cowpeas at high temperatures may possibly be attributed in part at least to their prompt and vigorous germination and more rapid hypocotyl elongation at high temperatures as compared with the beans.

Rhizoctonia solani was pathogenic to the beans and cowpeas at the lower temperatures, and infected the cotyledons to a considerable extent.

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THE OLIVE KNOT DISEASE: ITS INCEPTION, DEVELOPMENT, AND CONTROL

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THE OLIVE KNOT DISEASE: ITS INCEPTION, DEVELOPMENT, AND CONTROL[1, 2]

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INTRODUCTION

In 1931 the College of Agriculture was asked to investigate the olive knot disease, caused by *Bacterium savastanoi* E. F. S., then becoming serious in various districts of the Sacramento Valley. Despite the excellent work of previous investigators, information on many cardinal points in the development of the disease was lacking; and no specific control measures, aside from the removal and destruction of knots, were known. A knowledge of the circumstances under which the knot passes from a rather innocuous, occasionally occurring disease into a widespread and destructive malady was considered important. Consequently, the several factors that might be instrumental in predisposing the host to attack and in favoring the inception and development of the disease were particularly studied. In addition, the possibility of control was considered. The control data, though admittedly not of sufficient extent or diversity to warrant detailed recommendations, are promising bases for trials in various localities.

Most of the work reported herein was done in orchards near Corning, California.

HISTORY OF THE DISEASE IN CALIFORNIA

In 1898 Bioletti[3, 4] reported finding the disease in Merced County and stated that it had been present since 1893. R. E. Smith[23] mentioned its prevalence in the Sacramento Valley in 1907. It did not, however, become serious until 1909, when Smith[24] stated that studies were being initiated. In 1912, Horne, Parker, and Daines[10] investigated a serious outbreak in Sacramento County. Then followed a period when no account of serious damage appears in the records except in isolated cases. One such outbreak developed in Butte County 10 or 15 years ago, although no published record shows how long this lasted or how severe it became. The disease is not known to have become common in Tehama

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County before 1929, the year when it reached severe proportions both there and in Butte County. It has subsequently become prevalent in all olive districts in the Sacramento Valley and in parts of the San Joaquin Valley, but has not been reported as serious south of the Tehachapi Mountains.

VARIETAL SUSCEPTIBILITY

Bioletti[4] reported that the Columbella olive appeared to be more susceptible to olive knot than the Mission. According to E. F. Smith[21] the Nevadillo Blanco and Manzanillo are more susceptible than the Mission. During the present work the Sevillano, Nevadillo Blanco, Manzanillo, and Ascolano have been more affected by the disease than the Mission. The trunks of Sevillano trees have been severely attacked while those of the Mission remained comparatively healthy, even though the disease occurred commonly in the tops. This difference in trunk susceptibility is due in part to the fact that large swellings, which are composed of numerous dormant buds, occur on the Sevillano, while the Mission trunks are generally smooth. These swellings, called "uovoli" by Bioletti[4], each year put forth many suckers. As the suckers emerge they split the outer bark, thereby enabling the bacteria to enter.

Although the Mission olive has proved the least susceptible in ordinary years, following the freeze of December 1932 it developed the disease severely in many cases. This, together with inoculation tests, shows that it is apparently no more resistant than other varieties once the bacteria enter the tissues.

The importance of olive knot in one locality may be influenced by the varieties grown. In one district, for instance, a large part of the acreage is planted to Sevillano trees, while in another district more Missions are grown. This may, in part, account for the fact that the disease did not become particularly serious in the latter district until after the freeze of December 1932, while it was widespread in the former as early as 1931.

IMPORTANCE OF INFECTION CENTERS

Since the bacteria persist in the knots from one year to the next, the disease may spread from any affected tree to others. In examining the reasons for a severe outbreak, one should consider the original source of infection—whether certain trees had harbored the disease for a long time, or whether it was introduced from some other locality. The evidence points to its existence in one district under observation for at least 15 years and not to its recent introduction from elsewhere, since little nursery stock has come into this district for several years, the local nurseries having supplied the demand.

In certain of the orchards under discussion diseased trees were recognized at least 10 years ago, and in such cases the bacteria spread from those earliest infected trees to adjacent trees. These centers are today distinguishable in many instances (fig. 1), there being only a few orchards (mostly young orchards) where the disease is uniformly distributed among all the trees. In other words, the severity of the disease is governed, to no small degree, by proximity to infection centers. Under the next heading this point will be brought out more clearly.

Before the next phase is discussed, however, the possible existence of the disease in other hosts will be mentioned. The information on this point falls into two categories: (1) hosts other than the olive susceptible to *Bacterium savastanoi,* and (2) similar maladies of hosts related to the olive. C. O. Smith[16], who gives the most extensive evidence concerning the first point, found that by inoculation, knots similar to those of olive were developed on Arizona ash (*Fraxinus velutina*), *F. floribunda,* and swamp privet (*Adelia acuminata*). Lesions, but no knots, developed on stems of *Osmanthus aquifolium* and fringe-tree (*Chionanthus virginica*); small galls did, however, appear on inoculated leaves of the latter. Doubtful results were obtained on privet (*Ligustrum ovalifolium*) and jasmine (*Jasminum primulinum*). Smith notes that symptoms resembling those on olive occurred only on those hosts most closely related to the olive. More recently[18] he has proved that *Olea chrysophylla* Lam. is susceptible to *B. savastanoi.* This host, closely related to *O. europea* L., has been introduced into this country from East Africa.

Two naturally occurring, tubercular diseases of hosts related to the olive are known, the oleander knot, and the ash knot. In 1908 E. F. Smith[19] reported failure to obtain infection of the oleander with *Bacterium savastanoi* and suggested that the oleander tubercle might be caused by *B. tumefaciens.* Later, Smith, Brown, and Townsend[22] stated that they believed *B. tumefaciens* bore no relation to the disease. In 1912, Tonelli[25] briefly described the oleander organism but did not name it. In 1926, Ferraris[8] named it *Bacterium tonellianum.* Later C. O. Smith[17], reporting pathogenicity and cultural studies with this organism, found that it was able to attack the olive but that *B. savastanoi* did not attack the oleander. Since the two organisms were very similar in culture, he regarded them as strains of the same species, and named the oleander organism *B. savastanoi* var. *nerii,* apparently not aware of Ferrari's earlier designation.

In England, Austria, Germany, France, and Italy, the European ash, (*Fraxinus excelsior* L.), is attacked by a bacterial disease that is manifested as a canker with some hypertrophy at the margins. In 1933 Brown[6] reported finding the disease on the European ash in Washing-

ton, D. C. Although the causal organism would also attack *Fraxinus americana* L., she was unable to infect either *F. excelsior* or *F. americana* with *Bacterium savastanoi*. In extensive cultural comparisons she found that *B. savastanoi* and the ash organism were similar. Certain consistent differences, however, led her to describe the ash organism as a variety of the olive organism, *B. savastanoi* var. *fraxini*, n. var.

Apparently, therefore, *Bacterium savastanoi* might be harbored in certain hosts closely related to the olive; but surveys around Corning have not shown its presence in these plants.

DISSEMINATION OF THE BACTERIA

Although many practical questions relating to the frequency of long-distance transmission of the bacteria remain unanswered, considerable observational and some experimental data have been collected on spread of the disease in the orchard. Before these data are reviewed, the literature will be cited.

Petri[13], in Italy, asserted that *Bacterium savastanoi* was constantly found in the intestinal tract of the olive fly larvae, *Dacus olea*. This insect does not occur in California. Horne, Parker, and Daines[10] made considerable advances in explaining how the disease was spread through the tree by showing that the bacteria were exuded as a slime to surfaces of knots during rains and were then washed downward, infecting other branches. These workers found no evidence that insects transmitted the disease, but they suggested that birds might carry the bacteria from tree to tree.

The present work has also failed to indicate insects as agents in spreading the bacteria. At the time the disease is most active, insects are hibernating and the few found are hiding in the crevices of the bark. No direct evidence has been obtained that birds carry the disease, although this is one possibility among many.

Pruners, on the other hand, may be instrumental in long-distance spread, since the pruning operations are usually carried out when pruning tools may easily become contaminated. Although E. F. Smith[21] found that the bacteria on agar plates were killed by 30-minute exposure to sunlight, two experiments in the present work showed that contaminated instruments may transmit the disease even after being exposed to direct sunlight for several hours. A number of teasing needles, having been dipped in a water suspension of the organism, were placed out-of-doors in direct sunlight, when the temperature ranged between 27° and 29° C (81°–84° F). At 15-minute intervals inoculations were made with these needles. Infection occurred even after the needles had been exposed for 3 hours. The bacteria on the underside of the needle were

protected to some extent; but they would find even more protection on pruning tools. As shown by a further series of tests, the bacterial exudate, which had been placed on glass slides and allowed to dry in an incubator with a temperature between 17° and 18° C, contained viable bacteria at the end of a week. Conceivably, during winter pruning operations, contaminated tools might harbor the bacteria for an equally long time.

Probably a common method of transporting the disease for long distances has been the shipment of nursery stock. This can take place even in the face of rigid inspection, since the knots may not be visible at the time. Infections occurring in mid-winter will not develop knots until spring. Trees bearing such infection may therefore be dug, pass inspection, and be planted before the disease is visible. In one case that came to the notice of the writer, the disease developed in a lot of young trees shipped from a distance of 350 miles. One can easily understand, accordingly, how the disease might have been introduced into California.

During this work, considerable experimentation has shown that the bacteria may be spread downward by rains. The bacterial slime became visible in the fissures of the knots 20 minutes after the knots were wet. In one experiment healthy trees were wounded and were placed under diseased trees. A fine spray of water, allowed to fall over the trees for 7 minutes, resulted in heavy infection. This experiment showed that abundant, viable bacteria were present within a few minutes after the knots were moistened. Young potted trees placed under diseased orchard trees in wet weather developed numerous knots, further demonstrating the presence of bacteria during rains.

According to isolation studies, bacteria were present in knots containing live tissue; but they were markedly less abundant in knots that had died, presumably from the freeze, during the winter of 1932–33. Knots on greenhouse trees, never wetted by rain, exuded large amounts of bacteria upon being moistened. Under orchard conditions, therefore, viable inoculum will undoubtedly be present during the first autumnal rain following the hot, dry summer.

Sufficient evidence is at hand to prove the downward spread of bacteria during rains, but comparatively little to show the frequency and extent of lateral dissemination. Presumably the transmission by birds and pruners will be limited only by the activity of these agencies. On the other hand, in certain orchards where pruning has been done annually, the distribution of the disease by this means often appears to be limited. One end of an orchard, for example, may have been diseased for several years, while the other end remains comparatively healthy. Surveys in many orchards have revealed a rather consistent tendency for the disease

to confine itself to spots. This fact, mentioned earlier, is exemplified by the orchard represented in figure 1. The trees at the center of this area are more severely affected than those at the periphery, a situation resulting from the earlier diseased trees' furnishing inoculum for surrounding

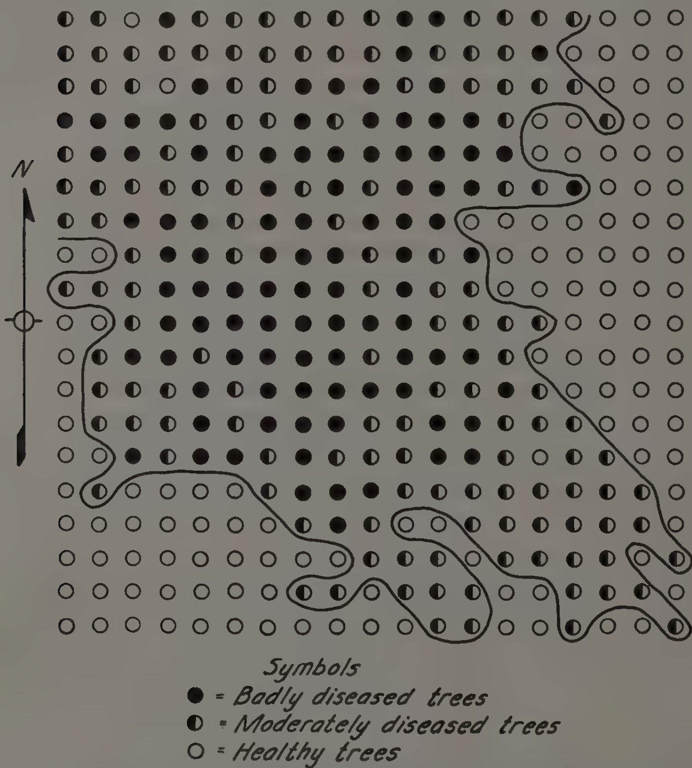

Fig. 1.—Distribution of infected trees in an orchard of Mission olives; mapped in 1932. The affected area is composed of a center of badly diseased trees surrounded by a zone of moderately diseased trees. This area is slightly longer in a north and south direction, the few remaining healthy trees being located on the east and west sides of the orchard.

trees. The extension of the diseased area during two years (1932 and 1933) (figs. 1 and 2) shows that the knot did not affect the entire orchard with uniform severity, even though 1932 was an epidemic year. This orchard, in common with others, contained affected areas somewhat longer in a north-south direction, the greatest number of healthy trees

being on the east and west sides. This phenomenon, often encountered, is considered strong evidence that the bacteria have been disseminated more freely in a northerly direction. Reasons for concluding that bacteria are carried farther to the north than to the south are given below.

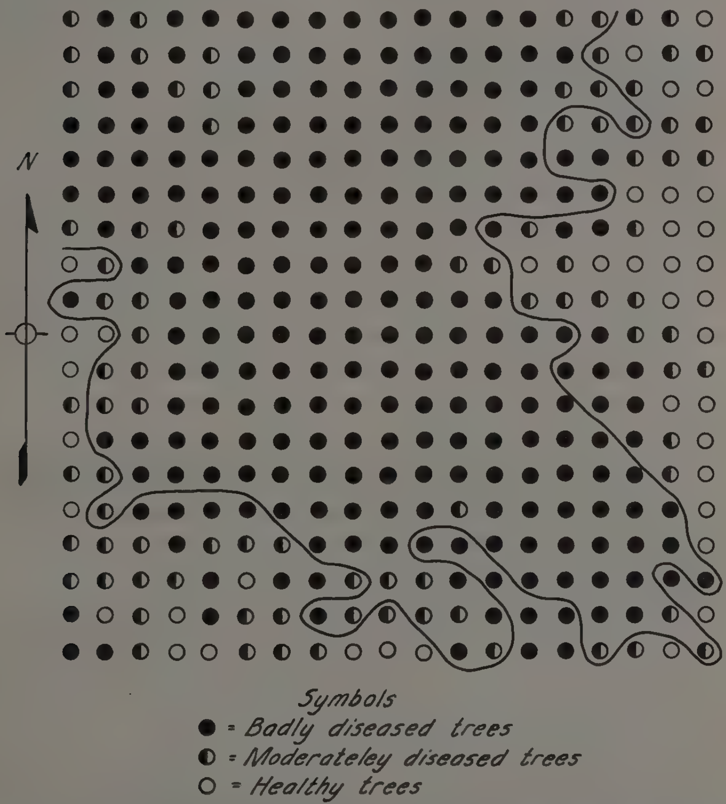

Fig. 2.—Same orchard as that in figure 1, mapped in 1934. The outline of the diseased area present in figure 1 is superimposed on this figure in order to compare the extent of spread between 1932 and 1934. The disease did not affect with uniform severity all of the orchard, even though 1932–33 was an epidemic year.

Figure 3 shows how the disease was distributed in an old grove of Mission olives. To the south of this orchard, separated by a road, is a block of badly diseased old trees; to the north, a younger block, also badly diseased. Several trees on the south end of this central group had become severely affected, while only a few scattered knots occurred in the

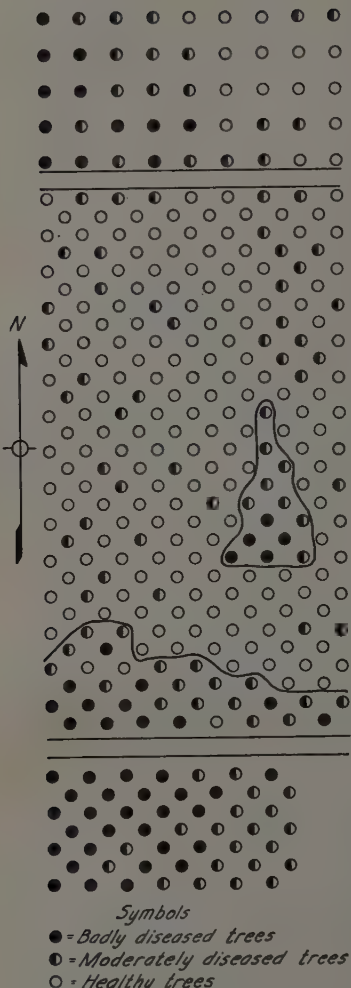

Fig. 3.—Spread of olive knot from one orchard to another. In the center orchard the disease had become more severe on the south end, 50 feet from badly diseased trees, than on the north end, 25 feet from a badly affected orchard. Note how the diseased trees are distributed in the small area near the center of the orchard.

trees along the north border, even though they were closer to badly diseased trees than those on the south. In the center of this orchard five trees had developed the knot severely. Although the disease had spread north of this area, adjacent trees to the south remained healthy.

In one grove, olive knot appeared for the first time in 1933. Only trees located in the northwest corner were affected. Knot had previously been present in the vicinity only on a few old trees, 100 feet to the southeast of this orchard. Apparently the bacteria had spread in a northwesterly direction but not far to the west.

All these observations indicate a disseminating agent that operated in a fairly constant manner. Wind or, more specifically, wind-borne rains appear to be the only factor that would do this. Since the bacteria are washed from the knots during rains, one may logically assume that wind might carry bacteria-laden particles of moisture for some distance. Two experiments have furnished some proof for this assumption. In one case potted healthy trees were wounded with a sterile knife and were placed 10 feet from the nearest diseased branches; in a second case, 40 feet to the north of diseased orchard trees. After a rainy period, accompanied by wind in each case, the trees were brought to Davis and placed in the greenhouse. The disease developed in several trees in

each instance. Although insects were not excluded from these trees, the experiments were conducted during mid-winter, when no insects were found active. Had they been responsible for carrying the bacteria, one would hardly expect the disease to develop only at wounds made with a sterile knife.

In rainy weather the prevailing winds are from the south or southeast. For example, during the two major infection periods of 1932–33 (December 17 to 23 and January 19 to 29), strong winds from the southeast occurred. The United States Weather Bureau (18 miles north of the experimental orchard) recorded a maximum wind velocity of 22 miles per hour on December 22, and 35 miles per hour on January 24. The potted trees, mentioned above as being located 40 feet to the north of the nearest diseased tree, were present in the orchard during the January infection period.

Although the possibility of dissemination of bacteria during dry weather is not entirely precluded, it does not seem likely to occur, inasmuch as a fresh supply of inoculum would not then be present on the surfaces of knots. Studies on this phase are planned for the near future.

INITIATION OF THE DISEASE

Time of Infection.—As shown by the foregoing discussion, *Bacterium savastanoi* is exuded when knots are wet. A supply of fresh inoculum will not be present on the surfaces of knots during the hot, dry summers. Infection, therefore, will probably not be common during the summer. To establish experimentally the time of infection during fall, winter, and spring, two methods have been employed. One consisted in making wounds at different times on healthy branches of diseased trees, thereby enabling the disease to start at these points. The second method consisted in placing wounded, young, healthy, potted trees under diseased orchard trees and at intervals of time replacing them with others. The potted trees, upon removal from the orchard, were brought to Davis, to avoid further exposure to infection. The first method merely indicated the occurrence or nonoccurrence of infection after the date of wounding and, to some extent, its abundance. The second method definitely established whether infection occurred during the period the trees were exposed in the orchard.

Since the distance to the experimental plots prevented a frequent change, the trees were left in the orchard over relatively long periods. The results, nevertheless, are useful when considered with those of greenhouse experiments. Figures 4 and 5 correlate the data collected by the second method with rainfall and temperature during 1932–33 and 1933–34. As the infection data shown in these figures represent the

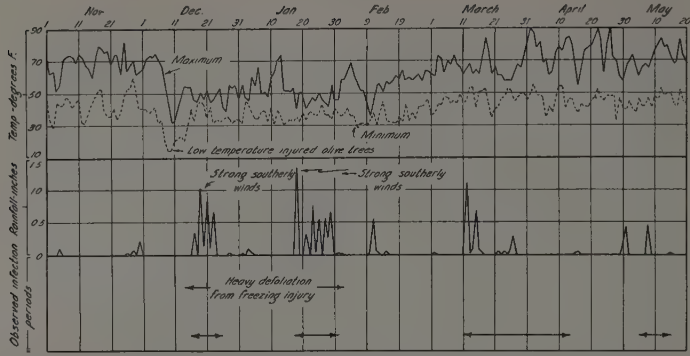

Fig. 4.—Temperature and rainfall during the winter of 1932–33 in relation to infection by *Bacterium savastanoi.*

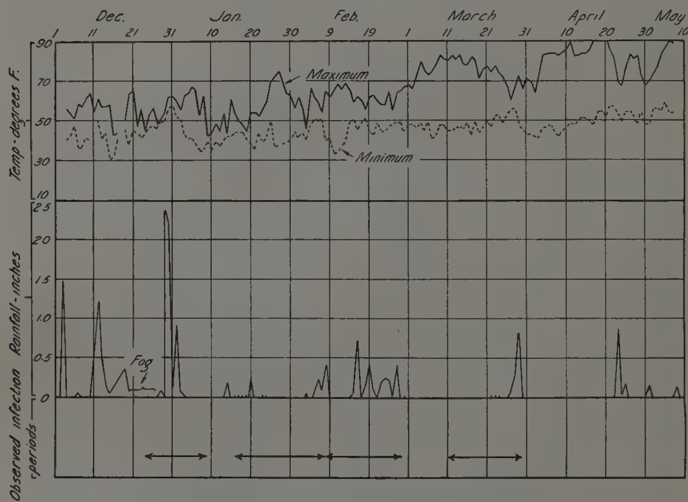

Fig. 5.—Temperature and rainfall during the winter of 1933–34 in relation to infection by *Bacterium savastanoi.*

periods during which trees were exposed and later became diseased, they do not necessarily mean that infection occurred throughout these periods. For example, the disease was probably initiated during the latter part of the last infection period shown in figure 5 and not during the early part.

According to these tests, infection occurred throughout the winter and spring. In 1933 the disease was initiated in blossom clusters as late

as May 16, during a rain that lasted only one hour. It was shown that the major part of infection during 1932–33 and 1933–34 occurred in December, January, and February, coincidentally with the longest rain periods of both winters. For instance, as mentioned earlier, during the winter of 1932–33 a greater part of the disease started in two periods—one from December 17 to 23, the other from January 19 to 29 (fig. 4).

Climatic Factors Favoring Infection.—In the dissemination of the disease, the importance of moisture in bringing the bacteria to the surface of the knots was apparent. Once the bacteria are spread over the surface of the tree, is any particular combination of temperature and moisture necessary for infection? If we study figures 4 and 5 carefully, we see that the disease was initiated under both winter and spring conditions. From December 16 to 26, 1932 (fig. 4), for instance, the mean daily temperature fluctuated between about 30° and 47° F. From May 6 to 16, on the other hand, the mean daily temperature was between 52° and 62° F. With trees in the greenhouse, it was shown that extremely high daytime temperatures will not preclude infection. On August 13, 1934, for instance, young olive trees were inoculated by placing the bacteria on cut ends of leaf peduncles. Within 14 days, definite symptoms had developed, although for 11 of these days the daytime tempertures went above 100° F (38° C), the average minimum temperature being 56° F. During fall, winter, or spring, therefore, the temperature is unlikely to be a limiting factor in infection.

Moisture, on the other hand, might conceivably be a limiting factor, for its absence would prevent movement and multiplication of the bacteria. Of necessity, only the moisture supplied from the outside can be considered, that supplied by the host tissue being an unknown variable. With a view to determining roughly the importance of moisture on the host surfaces to infection, two experiments were conducted. Young, potted trees were wounded in various ways with a sterile scalpel; *Bacterium savastanoi,* in water suspension, was atomized over the wounds; and the trees were placed in a chamber where the humidity was kept high enough to prevent drying and where the temperature varied between 15° and 20° C. At intervals of 1, 3, 5, 8, and 13 hours, three trees were removed from the chambers and were placed on greenhouse benches. All the trees developed knot, regardless of whether they were kept under humid conditions for 1 or for 13 hours. Infection of shallow surface wounds, however, made by lightly scraping the periderm or by cutting leaves from the twigs, appeared to require 3 or more hours of moisture. A later experiment did not entirely substantiate these results. In this experiment leaves were cut off close to the twig, and the bacteria were placed on the wounds. One series of twigs was enclosed in large test tubes

with moist, absorbent cotton for 5 days, and a second series was exposed to the greenhouse air. The air temperature did not go above 30° C for the first two days after inoculation. Enclosing the twigs in test tubes increased infection, since 93 per cent of such inoculations developed knots as against 68 per cent on twigs exposed to the greenhouse air. Apparently, therefore, rapid drying of the host tissue reduced infection but did not prevent it. Numerous inoculations by needle puncture have given uniformly high percentages of infection regardless of atmospheric humidity or moisture on the surface of the twigs. Bacteria that enter deep wounds would therefore be little affected by dryness of the atmosphere. Since conditions during winter and spring are not conducive to rapid drying of the trees, the bacteria would be able to infect during very short rains. Examples were afforded in 1933 when on May 8 and 16 *Bacterium savastanoi* was atomized over the surfaces of blossoms after the terminal blossom on each raceme had been removed. Light showers lasting only about one hour fell shortly after inoculation. About 15 per cent of the racemes developed the disease. An additional example of the ease with which infection occurs was cited earlier. A potted tree, which had been wounded by cutting pieces of bark from the trunk, was placed under a diseased tree; and water as a fine mist was sprayed over the two trees for 7 minutes, after which the inoculated tree was allowed to dry. Practically all the wounds became diseased.

INFECTION COURTS

E. F. Smith(21) states that wounds are necessary for infection. Although he had reference to stem infection, he failed in one experiment to obtain knots by spraying the bacteria on uninjured leaves. It has been commonly observed that wounds made by various agencies were infection avenues. Thus Bernès(2), Pagliano(11), Del Canizo(7), and Petri(14) have reported infection of wounds made by pruning tools, hail, frost, and wind-blown sand. In the present work, wounds made by pruning and cultivating tools were found to be commonly infected.

Probably the most important single factor in the severe development of the disease during 1933 was low temperatures of early December, 1932. As a result of this freeze numerous cracks developed in both large and small branches (fig. 6*A*) and the bark was loosened from the cambium for long distances above and below these cracks. In addition, the trees were badly defoliated; leaves were constantly falling throughout the latter half of December and throughout January. As noted earlier, two periods of rain, accompanied by strong winds, occurred from December 17 to 23 and January 19 to 29. The knots, in consequence, developed in such great numbers that entire tops of trees were killed (fig. 7).

Traveling in Italy in 1891, Pierce[15] noted that knots frequently developed at branch nodes. At first he thought that this came about through infection of the buds in the axils of leaves, but later he decided that the bacteria entered the terminal bud and that the knots developed as the

Fig. 6.—*A,* Knots beginning to develop in cracks caused by the freeze of December, 1932. *B,* Numerous knots not yet broken through the bark. The bacteria entered through minute cracks, caused by a freeze. The bark had separated from the wood in many places, enabling the bacteria to move freely along the cambium. *C,* Knots resulting from infection of leaf scars. *D,* Knots resulting from infection of frost cracks. The remains of the periderm covers some of the knots. Compare with *B. E,* Infection of blossom scars. Fruit had set in some cases. *F,* Infection of blossom scars. In cases where no fruit is set, the portion of the raceme distal to the knot withers, but the remainder persists for several months.

leaves appeared. Subsequently, however, Horne, Parker, and Daines[10] concluded that the knots at the branch nodes resulted from leaf-scar infection.

Bioletti[4] noticed knots appearing on the large swellings, or "uovoli," common on trunks of certain olive varieties. The knots were generally located at the bases of suckers arising from these areas. Horne, Parker, and Daines[10] noted infection of "growth cracks." The present writer has observed a similar situation. Suckers arising from the trunks break the outer bark as they emerge, leaving a crack through which the bacteria enter. Not infrequently, knots develop at the base of these suckers.

It is well known that the disease occurs also on the roots of the olive, although not so commonly as on aboveground parts.

Horne, Parker, and Daines[10] mention the common occurrence of knots at the scars produced by dropping of leaves. During the present

Fig. 7.—Severe disease in the top of a Sevillano olive tree. Note the distribution of knots at intervals along the branches. This is the result of leaf-scar infection. The tree has been greatly damaged by the disease.

investigation, these points were frequently infected (fig. 6*C*)—more commonly than any others, in fact, during the years when no freezes injured the twigs. For example, counts in one orchard during the spring of 1932 showed that as high as 90 per cent of the new knots on branches occurred at leaf scars.

An heretofore unreported infection of blossom racemes should be mentioned here (fig. 6*E, F*), showing that tissues exposed by the natural

abscission of organs other than leaves are avenues for invasion. Scars may be formed when individual blossoms drop from the raceme and when the entire raceme falls away. To distinguish them, the former are called blossom scars; the latter, raceme scars. Since raceme scars are, of course, formed in the axils of leaves, knots occurring at this point may appear to result from leaf-scar infection if the leaf has fallen. If a raceme sets fruit, it naturally persists until after the fruit is picked in the autumn, when it gradually withers and falls away, leaving a scar. Such natural breaks are designated fruit-stem scars to distinguish them from the raceme scars formed in the spring.

When knots were first found on racemes, blossom infection was considered possible. That it is unlikely is shown by the following experiment. Blossoms that had recently opened were sprayed with a suspension of *Bacterium savastanoi.* In one series the terminal blossom of the raceme was removed to provide an infection court; in a second series the racemes were left intact. Knots developed on about 15 per cent of the wounded racemes, whereas none occurred on the uninjured. According to figure 6 *E, F,* knots occurred regardless of whether the racemes had set fruit or not. In case no fruit is set, but a knot starts at one of the middle blossom nodes, the portion of the raceme distal to the tubercle withers and falls away, while the proximal portion persists. In other words, the developing knot prevents abscission of the raceme at the base, in this respect functioning as does a fruit. Assuming that direct blossom infection would affect the individual blossoms in the same way, some portion of this organ should be present in very young knots; but in no case has this condition been found, the first indication of infection being a slight swelling beneath the blossom scar. Apparently, therefore, raceme knots arise from entry of the bacteria into scars left by unset blossoms and not from entry into the blossom.

Infection of blossom and raceme scars will follow if rains occur while the blossoms are falling. Thus, in 1932, when the Sevillano olive was in full bloom on May 17, rains on May 21–22 resulted in infection of 30 per cent of racemes in certain trees. In 1933, on the other hand, no rain occurred after full bloom, and consequently no blossom or raceme-scar infection developed, even though rains did fall during the early part of bloom.

For some unknown reason, infection of fruit-stem scars has not been common. Two experiments showed that the fruit stems remain attached for 4 to 5 weeks after the fruit is picked. This fact may bear some relation to infrequency of infection.

FACTORS INFLUENCING THE FORMATION AND INFECTIBILITY OF ABSCISSION SCARS

According to observations presented under the preceding heading, every year a large part of infection in branches takes place through leaf scars, showing that infectible scars are present during the fall and winter. Consequently, the importance of susceptible tissue of this nature cannot be overemphasized. Defoliation during the winter of 1932–33 was responsible in no small measure for the fact that Mission trees, heretofore fairly free of the disease, became badly affected. Conversely, the comparative scarcity of infection courts during the winter of 1933–34 is the only known reason why new disease was rare in the spring of 1934, since the climatic conditions were shown to be extremely favorable. Any studies designed to determine the causes of epidemics must therefore consider the time and conditions under which the leaf scars are formed, together with the factors which determine the length of their infectibility. Blossom and raceme scars should also be considered, even though they are relatively less important than leaf scars.

TABLE 1

LEAF FALL IN SEVILLANO OLIVE TREES

Periods of leaf fall	Leaves off on branches produced in:		
	1929	1930	1931
	Per cent	*Per cent*	*Per cent*
Before January 7, 1932	57.0	17.6	0.0
From January 7 to May 17, 1932*	2.5	2.7	0.5
From May 17 to September 20, 1932	28.5	17.6	2.3

* These trees were in full bloom on May 17.

Since little appeared to be known concerning time of leaf fall in olive trees, studies were initiated in 1932. A series of branches with three years' growth were tagged in January, and a record was kept of the defoliation from each year's growth. The data in table 1 show only a small amount of defoliation between January 7 and May 17, whereas many leaves fell between May 17 and September 20. The heaviest defoliation occurred during and immediately after the blossoming period. The 1931 growth had lost no leaves before January 7, 1932, and only a few up to September 20, 1932; but the 1930 and 1929 growth had lost considerable foliage before January 7 and continued to lose it up to September 20. A series of 80 branches on 8 trees tagged in July, 1934, have been observed at monthly intervals. Considerable defoliation occurred

during July, August, and September but decreased in October. At the present writing (November), occasional yellow leaves may be found in the trees, though they are distinctly fewer in numbers than in October.

Although the defoliation just described can be considered a normal course, some variations within this cycle have been observed when conditions were unfavorable. Trees that suffered from lack of water late in the season lost considerable foliage in September and October. At such a time, and to a considerable extent during the spring, greater defoliation may occur on the south and southwest sides of the trees. Considerable variation in leaf fall between trees in the same orchard appears to result from differences in soil conditions. The orchards in the district under observation are located to a large extent on Tehama loam soils[9], which are rather compact and easily puddled. The subsoil, from 12 to 30 inches below the surface, is usually heavier and more compact than the top soil. Because of the compactness of soil and undulating topography, water tends to penetrate very slowly in certain places and rapidly in others; the low-lying areas are poorly drained, while others dry rapidly. The trees reflect these differences in their manner of growth. Where drainage is poor or where penetration of irrigation water is slow, the trees may suffer during the summer. As a result they may lose more leaves than trees in better locations. Observations in one orchard showed that on one-year-old branches, from which defoliation is usually light, the leaves off varied from 2.3 to 18.1 per cent in the case of trees in a low, wet place, and from 1.9 to 6.4 per cent in the case of trees in a better drained area. Further counts indicated that defoliation is heavier from short terminal than from long terminal growth. On the same tree, for instance, one-year-old terminal growth that was 6 inches or more in length had lost but 1.8 per cent of its leaves, whereas that shorter than 6 inches had lost 10.2 per cent. In other words, if a tree is affected in such a way that it produces a preponderance of short terminal growth, somewhat heavier defoliation will occur from the newer wood than where the terminal growth is greater. The instances cited above were by no means extreme, since in certain other orchards the sparsity of foliage in some trees, as contrasted with the abundance in others, was obvious.

The disposition of certain trees to show greater defoliation may partially explain the following irregularities in disease distribution: (1) that trees sometimes developed the knot badly on the south and southwest side but sparsely on the north side, and that (2) in certain cases the worst-diseased trees in an orchard were known to be at a distance from originally infected areas. The tendency for trees to lose more foliage on the south and southwest than on opposite sides would explain why the trees might develop knots most severely on these sides. Likewise, trees

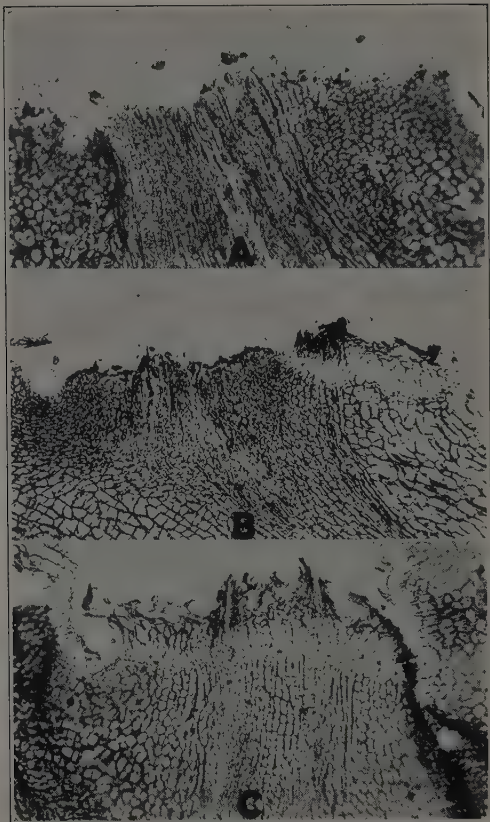

Fig. 8.—*A*, Leaf scar formed the day the fixation was made, showing the ragged ends of tissues. *B*, Blossom scar formed on May 5, fixation made on May 18. The xylem elements are still open at the scar. *C*, Raceme scar in the axil of a leaf. The blossoms on this raceme dropped about May 5; the raceme remained until the fixation was made on May 18, but fell upon being touched. A well-developed phelloderm is present. Compare with *B*.

that through unfavorable soil conditions are induced to drop more leaves during late autumn would be subjected to greater infection.

One point more might be mentioned regarding factors influencing leaf fall. In the spring of 1934 defoliation was greater on diseased branches than on healthy branches. Once a branch is diseased, it is weakened to such an extent that leaves are dropped, a condition which in turn in-

creases the avenues for entry of the bacteria. Under a later heading an apparent relation will be shown between the number of knots and the subsequent increase on uniform-sized limbs. As will be pointed out, several factors, including the effect of disease on leaf fall, might play equal parts in this phenomenon. That limbs might be predisposed to infection through the effects of the disease on defoliation seems to be a logical conclusion.

As shown by the preceding discussion, leaf fall may occur at almost any time of the year, but is most common in the spring and least common in the winter. This raises the question as to when the leaf scars are infectible and how long they remain so. Infection of scars of fruit stem, blossom, and raceme, being sporadic, is of less practical importance, but of great scientific interest. A detailed study is under way, beginning with the anatomical changes leading up to abscission of the leaf, and then following the development of phelloderm over the scar. Only a few preliminary observations on the latter phase are presented here.

Newly formed leaf scars, whether produced by artificial means, such as cutting off the leaf or allowing the soil to dry, or through natural defoliation, developed knots when inoculated. No information has been obtained on how long they remain infectible under a variety of conditions. In one experiment, leaf scars formed on potted greenhouse trees in November were inoculated the following March. No knots developed except at scars pricked by a needle before inoculation. Leaf scars formed during defoliation in the spring of 1934, together with blossom and raceme scars, were fixed and examined. Immediately after the leaf fell, the tissues at the scars were found to be torn, while the ends of the xylem elements were exposed and apparently open. Figure 8*A* shows a leaf scar the day the leaf fell; the ragged, exposed cells would seem to afford a foothold for the bacteria. Within a week, however, a well-developed phelloderm covered the scar. Figure 8 *B*, although picturing a raceme scar, adequately represents the situation in leaf scars. No phelloderm was present on the blossom scars, examined 13 days after blossom fall (fig. 8 *C*). If this condition is characteristic, these scars will probably remain infectible longer than leaf or raceme scars.

Although observations given above are preliminary to a more extensive study, certain tentative conclusions may be drawn. Judging from the rapid springtime development of phelloderm over leaf and raceme scars, these points remain infectible only a short while. Apparently, therefore, these scars are not avenues of entry the autumn following their formation. The one experiment where leaf scars did not become diseased when inoculated four months after formation would support this view. It seems likely that blossom scars might remain subject to

infection longer than either leaf or raceme scars. Assuming these conclusions to be essentially correct, those leaf scars which constitute the common infection courts, must be formed either during the winter when continued leaf fall would provide fresh, infectible tissues, or at an earlier period when phellogenesis is slow. The defoliation studies indicate only a slight amount of leaf fall during the rainy season. The exception, of course, was the winter of 1932–33 when heavy defoliation followed a severe freeze.

In conclusion it might be repeated that presence of infection courts, whether produced by artificial or by natural means, is one of the most important factors governing disease inception. The only apparent reason for sparsity of new knots during the season of 1933–34 was scarcity of infection courts. When these were produced in young potted trees, knot developed in abundance.

DEVELOPMENT OF SYMPTOMS

Although the first visible surface symptom of the disease is proliferation of tissue, removal of the branch periderm around a needle inoculation may reveal a water-soaking and apparent dissolution of tissue. These areas have at times become ¼ inch or more in length after midwinter inoculating of large limbs. Microscopic examination of infected young shoots show that considerable disorganization of the various tissues may occur before visible proliferation of cells begins. Such a disorganization is probably manifested as the water-soaking noticeable around inoculation points. A similar but more extensive symptom is present in the ash disease, caused by *Bacterium savastanoi* var. *fraxini*[6], where rather extensive canker formation is followed by proliferation of the tissues at the periphery.

The development of the knots depends upon growth of the host. Inoculations made in trees exposed to winter temperatures failed to produce knots until spring, although trees placed at temperatures favorable to tree growth produced visible knots within two weeks. No experiments have been performed at controlled temperatures, although inoculations at different times of the year have shown that knots will appear at temperatures much above the minimum for tree growth, provided the bacteria have become established in the tissue. As noted earlier, temperature probably does not limit infection during the ordinary season.

Under field conditions the development of knots has been followed rather carefully. In the winter of 1931–32, fifty branches on ten trees were examined at intervals. Aside from a few knots, probably overlooked during the first examination in the fall, no new development was recorded until April, after which they appeared in abundance. In a

similar series of branches observed during the winter of 1932–33, new knots failed to develop until late March, after which they appeared in such abundance as to prevent accurate counts. These observations agree with the experimental results, which showed that inoculations made in midwinter developed no symptoms until spring.

Certain investigators have described a metastasis, or development of secondary knots from migration of bacteria through the host tissue. According to E. F. Smith[21] (p. 389), "Deep tumors may also arise at a distance from the first tumor and these are due to bacteria which have mi-

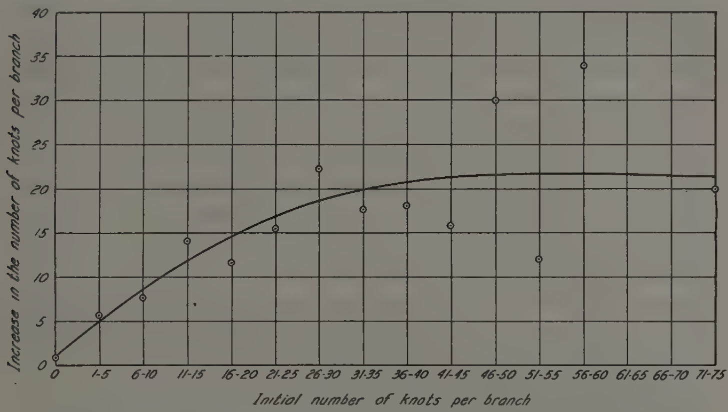

Fig. 9.—Relation of the initial number of knots to their subsequent increase on uniform-sized branches of olives.

grated from the primary tumor by way of the spiral vessels of the inner wood which in such cases are browned, more or less disorganized, and occupied by the gray-white slime of the bacteria." He[20] (Vol. 2, p. 71) further "observed numerous deep tubercles develop at a distance of 1, 2, and 3 feet from the point of inoculation within a period of 7 months in actively growing plants, both down and up the shoot."

According to Bonanni[5] the bacteria spread from the primary infection in the cortex to the wood, and finally, in the case of young twigs, to the pith. Upon reaching the wood an extensive diffusion of the organism through the vessels occurs, resulting in development of a knot at a distance from the primary infection. Should this phenomenon be common under field conditions, it would obviously complicate a control program. Once the branch is infected, there would be little hope of preventing subsequent disease development, either by excision of the primary knot or by spraying. Though the present work has not resulted in final proof one way or the other, the preponderance of evidence thus far has indi-

cated that new knots arising at a distance from primary infection can be traced in many cases, to entry of bacteria from the surface. In the field new knots may arise at or within a few inches of the base of old ones. Some of these may be metastatic tumors, although there is an equal likelihood that they result from surface infection. Considering the data in figure 9, we see an apparent relation up to a certain point between the initial number of tubercles on branches selected for uniformity in size, and the subsequent increase. There are three possible reasons: (1) the proximity of an inoculum source increases surface infection; (2) branches with large numbers of knots drop more leaves than those with fewer numbers, thereby increasing infection courts; and (3) the greater the number of knots the greater the frequency of "deep tubercle" development. The first two factors might conceivably operate in a complementary manner.

Although migration of bacteria through the vessels may result in secondary knots at or within a few inches of old infections, both field observations and some greenhouse experiments failed to show that they develop very far from the primary infection. In the first place, current or one-year-old branches are seldom attacked, even though they arise from badly diseased limbs. When the disease appears on one-year-old growth, it is usually located at leaf scars or at blossom and raceme scars. The current year's growth has not been observed to develop symptoms the year it is produced.

The greenhouse experiments have been even more convincing. Young, vigorously growing trees were on many occasions inoculated beneath the growing tip or at other places. Although some have been allowed to grow for two years, no disease appeared except at the point of inoculation. In one case a tree was inoculated at several places on the main stem about 6 inches below the growing tip. The knots at the inoculation points are now two years old and from 1 to 1½ inches in diameter, but no secondary knots have appeared. Meanwhile the tree has produced lateral branches 2 feet or more in length from points near the diseased area. In two experiments in the spring of 1934, young, vigorously growing branches were inoculated within an inch of the growing tip. These branches have since (four months later) grown from 18 to 20 inches without developing secondary knots. In all these experiments ordinary care has been taken to prevent water from being splashed over the trees, since, as was shown earlier, there is danger of spreading the bacteria to healthy parts even though the water comes in contact with the knots for only a few minutes.

EXPERIMENTS ON CONTROL

In the foregoing discussion an attempt has been made to bring into proper relation those factors which determine the feasibility of available control measures. Spread of the bacteria and initiation of the disease during the rainy season indicate the time during which certain preventive measures might be employed. Breaks in the natural protective layer of the host are infection courts; this fact and the occurrence of such infectible breaks in the form of abscission scars, pruning wounds, and growth cracks indicate the points at which preventive measures should be applied. The large number of quickly available, viable bacteria furnished by the knots already in the tree suggests removal of knots as a desirable method of control. Just how far each of these methods can be followed with beneficial results will be considered here.

Three possible approaches to control have been mentioned in the literature. The first, removal and destruction of knots, has been advised by most investigators interested in the problem; the second, increasing resistance of the trees to infection through fertilization of the soil, was suggested by Paoletti[12], who recommended the use of 2 to 4 kilograms of mineral superphosphates per tree and discontinuance of pruning operations; the third, applications of spray materials to prevent infection, was advocated by Bellini[1], who saw the necessity of protecting injuries produced by hail and who suggested bordeaux mixture as the preventive material. The present writer[26] obtained promising results with three applications of bordeaux. Paoletti[12] has also reported satisfactory control with 1 or 2 per cent bordeaux in four applications as follows: (1) after autumn rains begin, from September 1 to 15; (2) after picking the fruit, at the end of December; (3) at the end of February, to protect hail injuries; and (4) at the onset of the spring rains, from April 1 to 10.

A combination of the first and third method would seem advisable, inasmuch as the removal of knot alone would not insure against its return. Without entering into a discussion that could not be supported by much direct evidence, the writer would regard the second method of control—use of fertilizers to harden the tree tissues, thus making them less liable to injury and consequently to infection—as the least likely to succeed.

Removal and Destruction of Knots.—From a sanitation standpoint removal of all knots from a tree is a logical way of reducing infection; yet this method is limited in its application by the practical impossibility of removing all knots from badly diseased trees, and impaired in its usefulness because severe pruning opens new avenues for infection and

exposes large limbs to injury from sunburn. Actual attempts at a careful cleanup of diseased parts by a number of orchardists, furthermore, have demonstrated the inadequacy of this method; the disease reappeared the following year, in some cases with greater severity. There are two reasons for this: the opening of numerous new wounds by the pruning operations, and the impossibility of removing large numbers of knots from

TABLE 2

Results of Spraying Sevillano Olive Trees for the Control of Olive Knot, 1931–32

Treatments*	Increase of knots in three branches in each of 16 trees	
	Number	Per cent
Unsprayed	33	100
Homemade bordeaux, applications 1, 2, 3, 4	6	18
Homemade bordeaux, applications 2, 3, 4	6	18
Commercial bordeaux plus Volck, applications 2, 3, 4	10	30
Lime-sulfur, applications 2, 3, 4	19	57
Zinc-lime, applications 1, 2, 3, 4	15	45
Zinc-lime, applications 2, 3, 4	13	39
Sodium fluosilicate, applications 2, 3, 4	33	100
Unsprayed A†	27	100
Homemade bordeaux, application 3A	17	63

* Homemade bordeaux mixture, 4-4-50 (stone lime); commercial one-package bordeaux, 4-4-50, and 1 pint Volck per 9 gallons of spray; liquid lime-sulfur 1-40; zinc sulfate, stone lime, and water in the ratio of 4-4-50, respectively; sodium fluosilicate, a proprietary product used at the rate of 4 pounds per 50 gallons of water. Dates of spray applications: *1*, September 29; *2*, November 11; *3*, January 6; *3A*, February 25; *4*, March 29.

† The initial number of knots on these trees were counted at the time the application of homemade bordeaux *3A* was made, February 25.

limbs in such a way as to insure against their return and at the same time leave the limb undamaged.

In the face of these limitations the writer feels that, though destruction of all knots through pruning is to be desired, such a radical procedure must be subordinated to one less severe. Where drastic pruning is necessary to remove branches killed by the disease, or those so weakened by large numbers of infections that they are of no further use, a few large cuts to remove entire limbs would be preferable to numerous small cuts. The large cuts could then be covered with a bordeaux paste or some other good protective material.

Prevention of Infection by Sprays.—In the experiments of 1931–32[26] homemade bordeaux mixture, commercial bordeaux plus an oil emulsion (Volck), liquid lime-sulfur, zinc sulfate plus lime (zinc hydroxide) and sodium fluosilicate were tested comparatively. Table 2 gives the results of these tests; and figure 10 shows graphically the increase of disease in individual trees receiving bordeaux, zinc-lime, sodium fluosilicate, and

no spray. Homemade bordeaux appeared to be most efficient in preventing infection. Oil-bordeaux, lime-sulfur, and zinc-lime appeared to be distinctly inferior to homemade bordeaux, while sodium fluosilicate gave no evidence whatsoever of control.

Since bordeaux gave promising evidence of preventing infection, the work was expanded in 1932–33. A series of duplicate plots, receiving

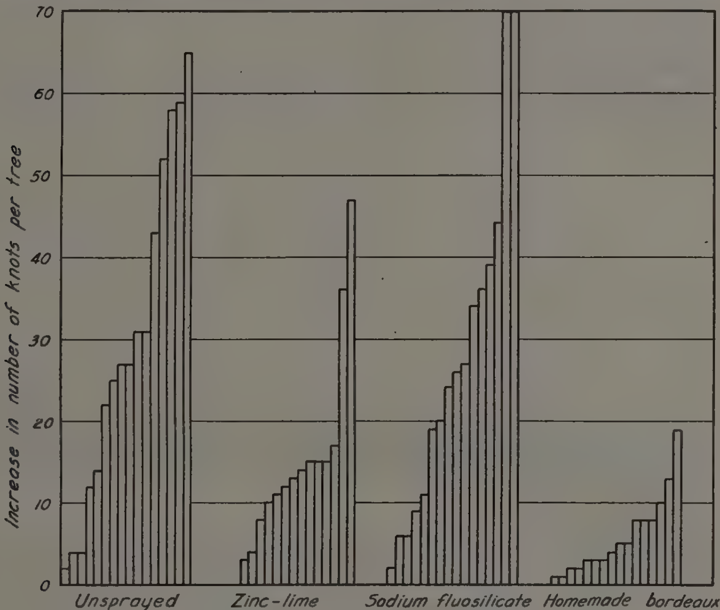

Fig. 10.—Comparison of the control obtained in individual trees receiving different spray materials.

different numbers of applications, was laid out in two districts. The freeze of December, 1932, however, rendered these tests useless, for some of the trees were killed outright, and most of them were severely injured.

In the spring of 1933 two experiments were performed to test the effectiveness of bordeaux in preventing infection of blossom racemes. At the time the blossoms were beginning to open, bordeaux mixture, 4–4–50, was applied to racemes on which the end blossom had been removed to insure an infection court. On a similar series no spray was applied. *Bacterium savastanoi* was then atomized over all of the blossoms in both series. Observations (table 3) made on June 13, before the unset racemes had fallen, showed that there was rather sparse infection of the unsprayed blossoms (15 and 5 per cent in the first and second experi-

ments, respectively) but that on August 15, 68 per cent of the unsprayed racemes in the first experiment, and 24 per cent of those in the second were infected, while the sprayed racemes developed only a few knots. Although the difference in percentage of infection between the June 13 and August 15 readings may have resulted in part from appearance of additional disease after the former date, the greater part of it was caused by the dropping of unset racemes, the total number of which had been used as a basis of computing the June 13 results.

Disease development in 1933–34 was very light. As mentioned earlier, this appeared to result from scarcity of infection courts and not from

TABLE 3

Control of Blossom-Scar Infection by Bordeaux Spray in May, 1933

Experiment		Total number blossom clusters sprayed	Percentage of blossom clusters that set fruit	Percentage of blossom clusters infected	
				June 13*	August 15†
First experiment	Unsprayed	326	44	15.0	68
	Sprayed	364	34	0.6	3
Second experiment	Unsprayed	396	48	5 0	24
	Sprayed	473	42	0.4	2

* Total racemes included in these counts.

† Only racemes that had remained were included; those that had failed to set fruit had, in most cases, dropped off. The differences between the June and August observations arise, therefore, from the dropping of unset blossom clusters, although a small difference may have resulted from development of new knots after the June observations.

unfavorable conditions for infection. An orchard of two-year-old trees sprayed with 4–4–50 and 8–4–50 bordeaux failed to develop sufficient disease for test purposes. In other orchards of older trees, where individual branches were sprayed, some data were obtained. In one experiment 30 branches on each of 3 trees were sprayed after 10 leaves had been removed from each branch. Bordeaux 4–4–50 and 8–4–50 were used in one orchard, and 6–6–50 was used in a second orchard. Table 4 shows the uniformly high degree of control obtained with all strengths of bordeaux used. In another experiment several materials were tested comparatively in two applications. The three new materials were used in concentrations that would give the copper equivalent of 4–4–50 bordeaux. The results (table 5) show that bordeaux mixture afforded somewhat better control than copper ammonium silicate and copper resinate and decidedly better control than basic copper sulfate. Application *3* was of very little benefit, since a large part of the infection occurred before it was made. A decided increase in control followed an application of bordeaux on February 7 (application *2*), a fact that emphasizes the need for renewing the spray material at intervals during the winter.

TABLE 4

RESULTS OF SPRAYING FOR THE CONTROL OF LEAF-SCAR INFECTION, 1934

Variety, orchard, and treatment*		Tree number	Percentage leaf-scars infected
Sevillano (Sloan) orchard	Unsprayed	1	27
		2	18
		3	10
	Homemade bordeaux, 4-4-50	1	0
		2	4
		3	1
	Homemade bordeaux, 8-4-50	1	5
		2	0
		3	3
Mission (Heinz) orchard	Unsprayed	1	29
		2	12
		3	14
	Homemade bordeaux, 6-6-50	1	3
		2	3
		3	0

* Spray applied February 7.

TABLE 5

RESULTS OF SPRAYING MISSION OLIVES WITH DIFFERENT MATERIALS FOR THE CONTROL OF OLIVE KNOT, 1933–34

Treatment*	Average increase in number of knots per ten branches	Percentage increase
Unsprayed	13.7	100
Homemade bordeaux (4-4-50), applications 1, 2, 3	2.0	14
Homemade bordeaux (4-4-50), applications 1, 3	4.3	31
Copper ammonium silicate†, applications 1, 3	6.3	45
Copper resinate†, applications 1, 3	6.3	45
Basic copper sulfate†, applications 1, 3	8.7	63

* Applications: *1*, November 24; *2*, February 7; *3*, February 27.

† Copper ammonium silicate (Copocil), manufactured by the California Spray Chemical Co.; copper resinate in an emulsifiable pine oil (Palustrex sulfonate), manufactured by the Wood Chemical Co., Jacksonville, Florida; basic copper sulfate, a so-called basic copper sulfate, manufactured by Marsh Brothers, Oakland, California.

Throughout the work a careful watch has been kept for signs of injury from the spray materials. No injury occurred until the winter of 1933–34, when applications of bordeaux on November 24 caused some defoliation of young trees. This injury is apparently produced only under certain weather conditions, since no injury followed an application on January 30. Rains, beginning on December 10, were followed by fogs that kept the foliage wet almost continuously throughout the rest

of December and most of January. By January 23, after two or three days of sunshine and somewhat higher temperatures, a few trees had lost some foliage. By January 30 increased defoliation was evident. There was no noticeable difference in defoliation between trees receiving 8–4–50 bordeaux and those receiving a 4–4–50 strength. The loss of foliage, though not great enough to injure the trees, was important in that it opened new avenues for infection. Further observations are necessary before the frequency and extent of injury can be ascertained. Judging from past experience, however, defoliation will be of only occasional consequence.

As shown in the third column of table 3, bordeaux mixture 4–4–50 apparently reduced the set of fruit when applied just prior to full bloom. This is of no practical importance since, if sprays are ever used to control blossom-scar infection, they will be applied when the unset blossoms are falling.

SUMMARY AND CONCLUSIONS

Olive knot, caused by *Bacterium savastanoi* E. F. S., has developed in California within the past five years from a level of little importance to one of great destructiveness. Although the disease has been present in the state since 1893, some of the olive districts have not heretofore experienced a serious outbreak.

Starting as scattered infections in a few trees, the disease spread to adjacent trees, thus gradually enlarging the affected areas.

The possibility that hosts other than the olive might be harboring the disease is considered unlikely, since none of these hosts were found affected near the localities under observation.

No commercial olive variety has proved immune to olive knot. The Mission, heretofore the least affected, became badly diseased following a freeze in December, 1932. The Sevillano, Nevadillo Blanco, and Manzanillo varieties are highly susceptible.

Dissemination of the bacteria for long distances may be accomplished by shipment of nursery stock and by pruners moving from orchard to orchard. Some experiments have shown that at moderately low temperatures and under dry conditions the bacteria may survive in the exudate for several days.

No evidence has been found that insects commonly transmit the disease.

Confirming earlier work, experiments showed that the bacteria are exuded to the surface of knots and are spread downward by rains. The exudate may become visible within a few minutes after the knot is wet. Further experiments showed that bacteria escaped from the knots and

infected healthy limbs below when a fine mist of water was directed over experimental trees for 7 minutes. Bacteria were abundant in knots with live tissue but were markedly less abundant in knots which had died recently.

In a number of orchards the lateral distribution of the disease is apparently more rapid in a northerly direction. The observational and experimental evidence suggests that wind-borne rain may be the responsible agent, since the prevailing winds during rainy weather are from the south and southeast.

A detailed field study showed that the disease is initiated during almost any rainy period but that the greatest amount of infection occurred during the longer rains of midwinter. Greenhouse studies indicated that temperature, within the range encountered during most winter and spring seasons, probably will not limit infection. Further tests indicate that, although it is not necessary for the surface of trees to be wet after inoculation of deep wounds, a rapid drying reduced, though it did not prevent, infection of shallow wounds.

Wounds of some sort are apparently necessary for infection of branches. During this work, freezing injuries, pruning wounds, and bark cracks produced by emergence of suckers proved to be common avenues for entry of the bacteria. In addition, scars produced by the dropping of leaves, individual blossoms, and racemes were attacked. Since, during ordinary years, the greatest amount of new knot on branches develops at leaf scars, the time and conditions governing formation of these scars were studied. These studies indicate that drought and the disease itself may increase defoliation and consequently infection.

According to preliminary microscopic studies of phellogenesis at leaf scars, those formed during the spring are not infectible the following autumn. Apparently, therefore, such leaf scars as are attacked by the bacteria during winter must be formed either during the winter, when continued leaf fall would provide fresh, infectible tissue, or at an earlier period when phellogenesis is slow.

Under field conditions, external symptoms in the form of knots did not develop during the winter. Even when infection occurred in December, the knots did not appear until spring. Trees placed under temperatures favorable to growth, however, developed visible symptoms in two weeks, merely demonstrating the fact that growth of tubercles depends on growth of the host, which, in turn, depends upon temperature.

Consideration has been given to the importance of metastasis, or formation of secondary knots resulting from migration through the host tissue of bacteria from a primary infection. Both observations and ex-

periments tended to show that the development of secondary symptoms at a distance from primary infections is not of common occurrence.

The important reasons for the recent widespread outbreak in the Sacramento Valley appear to be: (1) the presence of the disease in most localities for a number of years; (2) the presence of highly susceptible olive varieties, such as Manzanillo and Sevillano; (3) the dissemination of the bacteria by pruners, by wind-borne rain, and, to some extent a few years ago, by diseased nursery stock; (4) and a freeze during December, 1932, resulting in splitting of the bark and defoliation during the rainy season.

Studies on control have dealt largely with prevention of infection by spray applications during fall, winter, and spring. The following spray materials have been tested: homemade bordeaux mixture, commercial bordeaux plus a spray oil, zinc sulfate plus lime, lime-sulfur, sodium fluosilicate, copper ammonium silicate, copper resinate in an emulsifiable pine oil, and a so-called "basic copper sulfate." Homemade bordeaux mixture, in strengths of 4–4–50, 6–6–50 and 8–4–50, prevented infection to a considerable degree. All of the other materials, with the exception of sodium fluosilicate, reduced infection somewhat, but none appeared to be so effective as bordeaux.

Although further work is necessary to determine the number of applications necessary for the most efficient control, such data as are available indicate that the spray must be renewed at different times during the winter and spring, the first application being made in the fall before the rains begin.

Defoliation occurred to a moderate extent following an application of bordeaux 4–4–50 and 8–4–50, prior to a prolonged period of rains and fog. A second spray, however, applied after this wet period, produced no injury. The damage from this cause is not considered great enough to warrant objections to the use of bordeaux.

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